Equivocal Colonic Carcinogenicity of *Aloe arborescens* Miller var. *natalensis* Berger at High-Dose Level in a Wistar Hannover Rat 2-y Study

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ABSTRACT: A 2-y carcinogenicity study of Aloe, *Aloe arborescens* Miller var. *natalensis* Berger, a food additive, was conducted for assessment of toxicity and carcinogenic potential in the diet at doses of 4% or 0.8% in groups of male and female Wistar Hannover rats. Both sexes receiving 4% showed diarrhea, with loss of body weight gain. The survival rate in the 4% female group was significantly increased compared with control females after 2 y. Hematological and biochemical examination showed increase of RBC, Hb, and Alb in the 4% males. The cause of these increases could conceivably have been dehydration through diarrhea. AST and Na were significantly decreased in the males receiving 4%, and Cl was significantly decreased in both 4% and 0.8% males. A/G was significantly increased in the 4% females, and Cl was significantly decreased (0.8%) in the female group. Histopathologically, both sexes receiving 4% showed severe sinus dilatation of ileocecal lymph nodes, and yellowish pigmentation of ileocecal lymph nodes and renal tubules. Adenomas or adenocarcinomas in the cecum, colon, and rectum were observed in 4% males but not in the 0.8% and control male groups. Similarly, in females, adenomas in the colon were also observed in the 4% but not 0.8% and control groups. In conclusion, Aloe, used as a food additive, exerted equivocal carcinogenic potential at 4% high-dose level on colon in the 2-y carcinogenicity study in rats. Aloe is not carcinogenic at nontoxic-dose levels and that carcinogenic potential in at 4% high-dose level on colon is probably due to irritation of the intestinal tract by diarrhea.

Keywords: *aloe arborescens* Miller var. *natalensis* berger, Kidachi aloe, no observed adverse effect level, Wistar Hannover rats, 2-y carcinogenicity study

Introduction

Aloe arborescens Mill var. *natalensis* Berger (designated as "Aloe"), a member of the family *Liliaceae*, is called Kidachi Aloe in Japan, where it is included in the list of existing food additives, developed by the Standards and Evaluation Div., Dept. of Food Safety, Ministry of Health, Labour and Welfare. Aloe contains barbaloin and aloenin as major components (Kuzuya and others 2001) and has long been used as a medicine for treatment of skin injuries and burns (Grindlay and Reynolds 1986; Koike and others 1995). Various pharmacological and therapeutic activities have been studied, and have been reports of anti-inflammatory effects (Fujita and others 1976), purgative action (Akao and others 1996), antidiabetic influence (Beppu and others 2003; Beppu and others 2006), and antitumorigenic effects of extracts (Shimpo and others 2002, 2003). Recently, Shimpo and others reported mild suppressive effects of Aloe arborescens on azoxymethane-induced intestinal carcinogenesis in the colon (Shimpo and others 2006) and inhibition of DNA adduct formation (Shimpo and others 2001). A certain amount of Aloe consumption by humans is expected from its pharmacological use by the oral route. Since administration may be long term, toxicity and carcinogenicity assessment is required.

In a previous 90-d subchronic toxicity study of F344/DuCrj rats administered diets containing 0%, 0.25%, 1%, 4% Aloe, diarrhea, decreased body weight gain, and increase of leucocytes were evident in 4% males and females (Kamiya 2002). We also conducted a 1-y chronic toxicity study using Wistar Hannover rats at dietary concentrations up to 4% (0%, 0.16%, 0.8%, and 4%) to more fully evaluate the potential toxicity of Aloe with 1.91% aloenin and 0.83% aloin. From this experiment, we concluded the number of observed adverse effect level for Aloe to be 0.16% in the diet, which is equivalent to 87.7 and 109.7 mg/kg/d in male and female Wistar Hannover rats, respectively (Matsuda and others 2007).

Here, a 2-y carcinogenicity study of *Aloe arborescens* Miller var. *natalensis* Berger in Wistar Hannover rats was employed to provide a more reliable assessment of long-term toxicity and carcinogenic potential.

Materials and Methods

Chemicals

Whole leaf powder of Aloe, the same grade as used as a food additive, was obtained from Nippon Funmatsu Yakuhin (Osaka, Japan) mixed at concentrations of 0.0% (control), 0.8%, and 4% into powdered basal diet MF (Oriental Yeast Co. Ltd., Tokyo, Japan), and pelleted. The concentrations of aloenin and aloin (barbaloin and isobarbaloin), the major effective elements of Aloe, in both the

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whole leaf powder (Aloe powder) and the pelleted diet for this study were measured and evaluated using high-performance liquid chromatography (HPLC) by Nippon Funmatsu Yakuhin, after storage at room temperature for 2 wk (Table 1). Recapture rates for aloenin in the admixed diet at each concentration level were confirmed to be 72% to 87%.

Experimental animals and housing conditions

A total of 156 male and 162 female Wistar Hannover rats, purchased at 5 wk of age from CLEA JAPAN Inc. (Tokyo, Japan), were maintained in Kagawa Univ. Animal Facility according to the Institutional Rules for Animal Experimentation. The Animal Care and Use Committee for Kagawa Univ. approved the protocol of this experiment. The animals were acclimated for 1-wk prior to the commencement of the experiment, when they were randomly allocated, 52 to 58 animals/sex/group and maintained in a room with a barrier system under constant conditions: temperature of 24 ± 2 °C, relatively humidity 60% ± 10%, and 12-h light/dark cycle. They were housed 2 or 4 rats per cage, males at 2 rats per cage, and females at 4 for reasons of body sizes. They were given free access to tap water and diet throughout.

Experimental design

The animals received Aloe at concentrations of 0% (control), 0.8%, or 4% for 2 y. During the treatment period, general conditions were examined daily. Body weights and food consumption were recorded once weekly during the first 3 mo of the administration period, and once every 4 wk thereafter. After the treatment period of 2 y, blood was collected from all survivors at necropsy under deep anesthesia. Hematological examinations were performed at SRL Inc. (Tokyo, Japan) for the following parameters: red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (Pt), white blood cell count (WBC), total protein (TP), albumin (Alb), albumin/globulin ratio (A/G), total bilirubin (T-bil), glucose (Glu), total cholesterol (T-cho), triglyceride (TG), phospholipids (PL), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), inorganic phosphorus (IP), sodium (Na), potassium (K), chloride (Cl), aspirate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and γ-glutamyltranspeptidase (γ-GTP). All animals were subjected to complete necropsy and the brain, lungs, heart, spleen, thymus, salivary glands, liver, adrenals, kidneys, and testes/ovaries of each animal were removed and weighed. For salivary glands, adrenals, kidneys, testes, and ovaries, weights for each side were separately recorded and the totals were then used for calculation of group mean and standard deviation (SD) values. In addition to these organs, portions of the nasal cavity, trachea, aorta, pituitary, thyroids, parathyroids, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, urinary bladder, epididymides, prostate, seminal vesicles, ovaries, uterus, vagina, mammary glands, skin, submandibular and mesenteric lymph nodes, thymus, sternum, femur (including bone marrow), sciatic nerve, spinal code (thoracic and lumbar code), eyes, Harderian glands, and thigh muscle were excised and specimens were fixed in 10% buffered formalin. After the fixation, the pituitary, prostate, seminal vesicles, and uterus were weighed. All of the excised organs and tissues were routinely processed for embedding in paraffin, sectioned at 4 mm, and stained with hematoxylin and eosin for histopathological examination.

Table 1 — Concentration of aloin and aloenin from the whole leaf powder of Aloe and pelleted diet admixed with Aloe.

		Pelleted diet		
	The whole leaf powder of Aloe	0 (control)	0.8	4
Aloin	0.83 (%)	N.D.	0.0009	0.0179
Aloenin	1.91	N.D.	0.0022	0.0663

Aloin and aloenin were measured after storage at room temparature for 2 wk with use of 1 batch of Aloe.
N.D. = not determined.

Statistics

Variance in data for body weights, hematology, serum biochemistry, and organ weights was analyzed for homogeneity by the Bartlett test. When the data were homogenous, one-way analysis of variance (ANOVA) was used. In the heterogenous cases, a Kruskal–Wallis test was performed. When statistically significant differences were indicated, Dunnett's multiple test was performed for comparison between control and treated groups. The incidences of tumors, preneoplastic, and nonneoplastic lesions were analyzed with the Fisher's exact probability test or the Mann–Whitney's U-test.

Results

Survival rates, general condition, body weight, and food consumptions

The survival rates for rats given 4%, 0.8%, or 0% Aloe for up to 2 y were 79%, 77%, and 67%, respectively, for males and 77%, 67%, and 59% for females (Figure 1). The survival rate of 4% female group was significantly increased compared with control female group and that of 0.8% female, 4% and 0.8% male groups were also elevated but without any significant difference. Regarding the general condition, males of the 4% group suffered from diarrhea from 1 wk after the commencement to the end of study, and the 4% females showed a tendency for loose stools. No other obvious findings were observed. Body weights showed a significant decrease in both male and female 4% groups (Figure 2 and Table 3), and showed a tendency for decrease at 0.8% group. Food consumption showed no significant change in any group (Table 2).

Organ weights

Relative weights of organs are shown in Table 3. Relative weights of the liver were significantly increased ($P < 0.05$) in the male 4% group. Absolute (not shown in Table 3) and relative weights of the spleen were significantly increased ($P < 0.05$) in the males of both 4% and 0.8% groups but did not show a dose response. Relative uterine weights were significantly increased ($P < 0.05$) in the females given 4% group.

Hematology and serum biochemistry

Results of hematology and biochemistry are summarized in Table 4. Hb and Pt were significantly increased in the male 4% group. On the other hand, female rats showed no significant change compared with controls. Regarding biochemical data, AST and Na were significantly decreased in the males given 4%, and Cl was significantly decreased in the males at both 4% and 0.8%. A/G was significantly increased in the 4% females, and Cl was significantly decreased in the female 0.8% group. Some slight changes were found in other parameters, but there was no dose response.

Necropsy and histopathology

Due to the deaths and autolysis during the experimental period, necropsy could only be performed on 38 to 45 rats in each group at

the end of the experiment. Macroscopically, in the male and female 4% groups (especially in males), some ileocecal lymph nodes appeared swollen. There were no other macroscopic findings observed.

Table 5 summarizes data for incidences of the nonneoplastic or preneoplastic lesions for which significant intergroup differences or high frequency were observed. The incidences of severe dilatation of the mesenteric lymph sinus were significantly

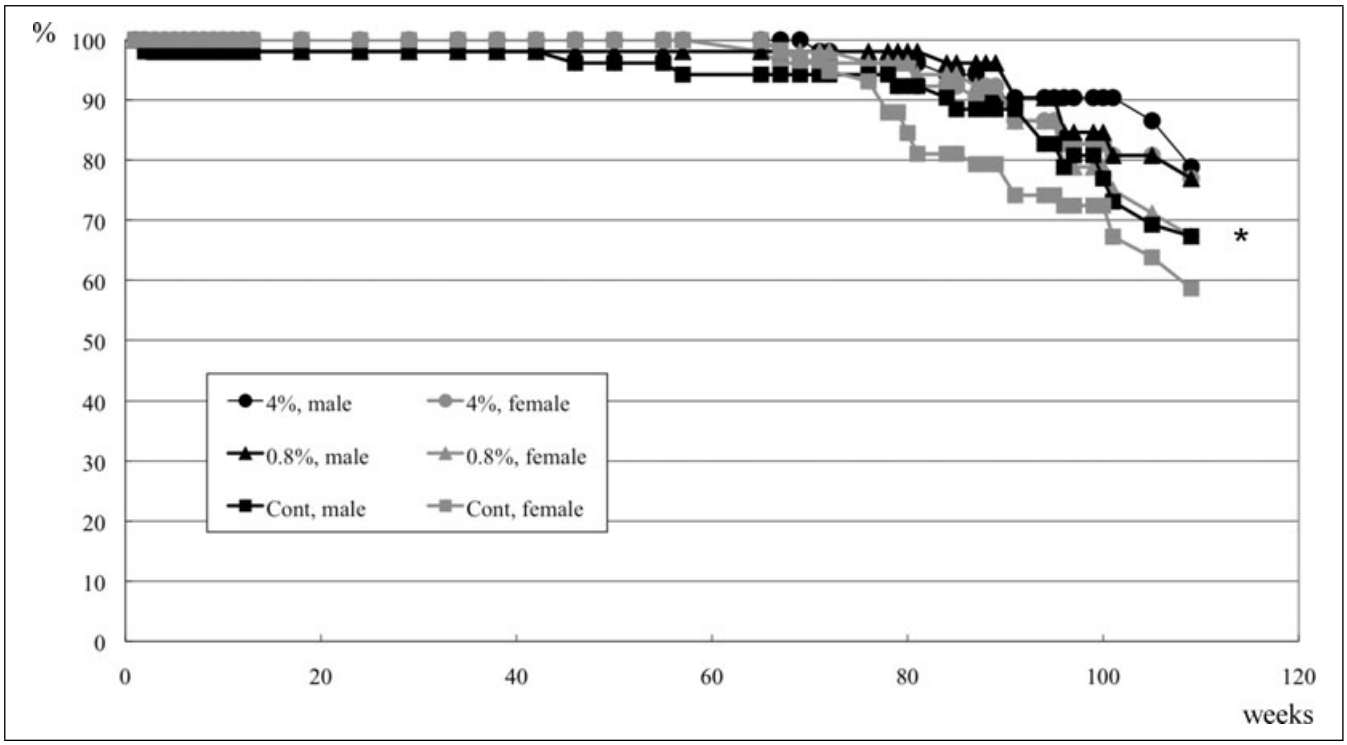

Figure 1—Survival rates of Wistar Hannover rats over 2 y. *$P < 0.05$ in the 4% female group compared with the control female group.

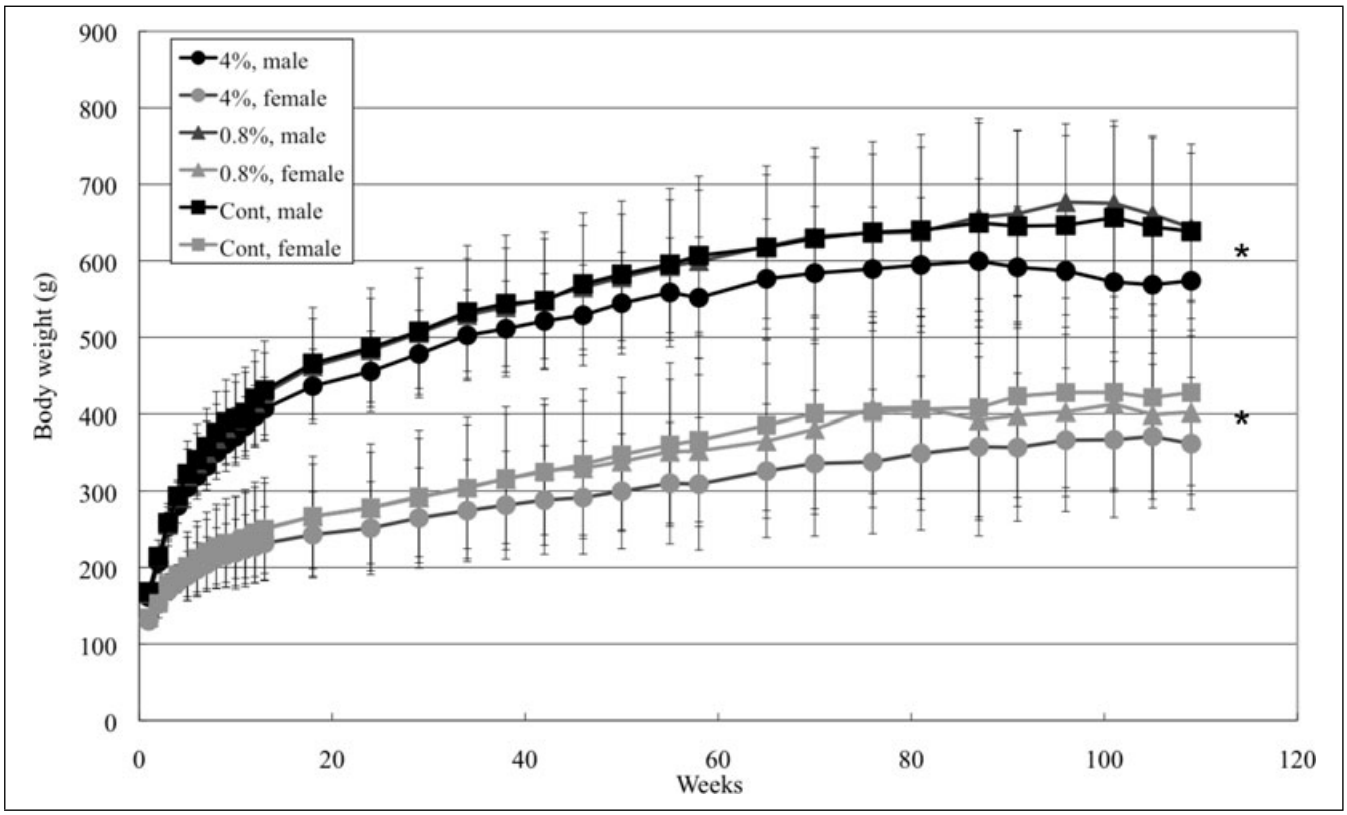

Figure 2—Growth curves of Wistar Hannover rats over 2 y. *$P < 0.05$ in both male and female 4% groups compared with control male and female groups, respectively.

Table 2 — Average intakes of food and Aloe extract per rat.

	Dose of Aloe (%)	Daily intakes		Total intakes of Aloe (g/kg b.w./2 y)
		Food (g/kg b.w./d)	Aloe (mg/kg b.w./d)	
Male	0.0	47.4 ± 18.4	N.D.	N.D.
	0.8	46.8 ± 19.1	374.5 ± 152.7	272.6 ± 111.1
	4.0	51.8 ± 18.3	2073.9 ± 731.9	1509.8 ± 532.9
Female	0.0	59.0 ± 18.4	N.D.	N.D.
	0.8	57.9 ± 17.4	462.9 ± 138.8	337.0 ± 101.0
	4.0	62.7 ± 17.5	2509.6 ± 701.7	1827.0 ± 510.8

N.D. = not determined.

elevated in the 4% males ($P < 0.01$), 4% females ($P < 0.01$), and 0.8% males ($P < 0.05$) as compared to the controls. Yellow-brown pigmentation in the ileocecal lymph nodes was found at significantly elevated incidence in the 4% males ($P < 0.01$) and females ($P < 0.01$) (data not shown). No tumorous changes of lymph nodes were observed in any group. Thickening of epithelium of the colon was also found to be significantly elevated in incidence in the 4% and 0.8% male and 4% female groups ($P < 0.01$) as compared to the controls. In the kidneys, chronic nephropathy was significantly more frequent in the female 4% group ($P < 0.05$) as compared to the controls. Yellow–brown pigmentation in renal tubules was found at significantly elevated incidence in the 4% and 0.8% males ($P < 0.01$) and females ($P < 0.01$). Though not listed in Table 5, nonneoplastic or preneoplastic

Table 3 — Body weight and relative weights of organs.

	Basal diet		0.8% Aloe		4% Aloe	
	Male	Female	Male	Female	Male	Female
(Number of rats)	34	33	37	33	40	39
Body weight (g)	600.5 ± 111.9	400.1 ± 88.4	610.7 ± 85.0	374.9 ± 97.8	536.4 ± 73.0[c]	337.0 ± 64.8[b]
Brain	0.43 ± 0.06	0.62 ± 0.11	0.38 ± 0.06	0.57 ± 0.15	0.48 ± 0.56	0.54 ± 0.15
Pituitary	0.01 ± 0.01	0.03 ± 0.04	0.02 ± 0.06	0.02 ± 0.04	0.01 ± 0.01	0.03 ± 0.04
Thymic gland	0.08 ± 0.14	0.06 ± 0.05	0.11 ± 0.19	0.24 ± 0.63	0.30 ± 0.83	0.26 ± 0.70
Lung	0.30 ± 0.04	0.34 ± 0.07	0.27 ± 0.04	0.32 ± 0.08	0.52 ± 1.38	0.37 ± 0.32
Liver	2.62 ± 0.43[a,c]	2.42 ± 0.46	2.30 ± 0.34	2.44 ± 0.46	2.26 ± 0.26	2.34 ± 0.56
Kidney	0.74 ± 0.25	0.84 ± 0.87	0.57 ± 0.12	0.66 ± 0.24	0.83 ± 1.07	0.65 ± 0.25
Spleen	0.25 ± 0.05[a]	0.28 ± 0.16	0.25 ± 0.10[a]	0.30 ± 0.12	0.46 ± 0.70	0.25 ± 0.12
Uterine		0.28 ± 0.17[b]		0.26 ± 0.12		0.19 ± 0.08
Ovary		0.16 ± 0.71		0.04 ± 0.02		0.10 ± 0.28
Prostate	0.30 ± 0.10		0.25 ± 0.14		0.26 ± 0.14	
Testis	0.71 ± 0.12		0.61 ± 0.10		0.83 ± 1.49	

[a] $P < 0.05$ compared with male basal-diet group.
[b] $P < 0.05$ compared with female basal-diet group.
[c] $P < 0.05$ compared with male 0.8% Aloe group.

Table 4 — Hematological and biochemical data of Wistar Hannover rats.

		Basal diet		0.8% Aloe		4% Aloe	
		Male	Female	Male	Female	Male	Female
WBC	/μL	6379.4 ± 2847.6	4306.7 ± 1800.4	6905.4 ± 2608.8	4381.8 ± 2465.2	6270 ± 2727.5	4124.3 ± 2856.4
RBC	$\times 10^4$/mm^3	691.4 ± 158.5	684.3 ± 113.9	738.1 ± 99.4	643.2 ± 118.8	765.8 ± 95.9	738.4 ± 75.2[d]
Hb	g/dL	11.7 ± 3.5	13.2 ± 2.7	12 ± 2.5	12.2 ± 2.8	13.4 ± 1.8[a]	14.5 ± 1.2[d]
Ht	%	38.3 ± 10	42 ± 7.6	40.1 ± 6.6	38.7 ± 8.3	43.5 ± 5.3	45.3 ± 4.1
MCV	μ3	55.1 ± 4.4	61.2 ± 4.2	54.2 ± 4.2	59.8 ± 3.8	57.2 ± 3.7[b]	61.5 ± 3.1
MCH	pg	16.6 ± 2.3	19.2 ± 2.1	16.2 ± 2.2	18.8 ± 1.8	17.6 ± 1.4	19.7 ± 0.8
MCHC	%	30.3 ± 2.6	31.4 ± 2.1	29.8 ± 2	31.5 ± 1.6	30.7 ± 1.2	32 ± 1
Pt	$\times 10^4$/mm^3	114.3 ± 24.8	99 ± 17.1	122.1 ± 30.2	108.6 ± 24.1	135 ± 24.6[a]	101.9 ± 16.5
TP	g/dL	6.4 ± 0.7	7.1 ± 0.8	6.6 ± 0.4	6.8 ± 0.6	6.4 ± 0.5	7.1 ± 0.4
Alb	g/dL	3.2 ± 0.6	4 ± 0.7	3.4 ± 0.3	3.8 ± 0.6	3.4 ± 0.5	4.3 ± 0.4[d]
A/G		1 ± 0.2	1.3 ± 0.4	1.1 ± 0.2	1.3 ± 0.4	1.1 ± 0.2	1.6 ± 0.3[c,d]
T-Bil	mg/dL	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0.1	0.1 ± 0.3	0.1 ± 0
γ-GTP	IU/L	2>	2>	2>	2>	2>	2>
AST	IU/L	99.5 ± 49	112.5 ± 105.2	68.6 ± 16.1	99 ± 51.3	60.3 ± 42.4[a]	76.5 ± 21.2
ALT	IU/L	31.2 ± 15.9	34.1 ± 28.8	26.4 ± 8.7	32.4 ± 21.5	30.6 ± 51.1	21.8 ± 8.6
ALP	IU/L	216 ± 87.2	97.3 ± 50.9	196.7 ± 50.7	115.8 ± 57.2	210.2 ± 130	142.9 ± 283.1
T-Cho	mg/dL	133.4 ± 63	109.6 ± 43.1	133.7 ± 73.8	102.1 ± 38.9	146.3 ± 65.5	94.9 ± 20.2
TG	mg/dL	137.1 ± 78.2	100 ± 108.6	185.5 ± 165.5	66.8 ± 57.3	175.3 ± 112.7	94.1 ± 78.5
PL	mg/dL	197.4 ± 66.4	188.8 ± 55.8	195.4 ± 85.2	174.6 ± 52.3	208.6 ± 78.8	173.8 ± 33.4
Glu(BS)	mg/dL	126.4 ± 28.5	119.6 ± 29.3	132.7 ± 19.5	122.1 ± 19	125.9 ± 21.2	119.9 ± 19.2
BUN	mg/dL	19.5 ± 7.9	15.3 ± 7.6	16.9 ± 8.2	15.4 ± 8.9	19.5 ± 13	14.3 ± 3.4
Cr	mg/dL	0.5 ± 0.4	0.3 ± 0.1	0.4 ± 0.3	0.4 ± 0.4	0.5 ± 0.6	0.3 ± 0
Na	mEQ/dL	142.7 ± 1.9	140.2 ± 3.1	141.5 ± 1.5	138.4 ± 3.6	139.9 ± 3.3[a]	139.6 ± 1.5
K	mEQ/dL	5 ± 0.7	4.7 ± 0.8	5.1 ± 0.6	4.6 ± 0.6	4.7 ± 0.6[b]	4.5 ± 0.6
Cl	mEQ/dL	107.7 ± 2	104.7 ± 3.2	105.5 ± 1.7[a]	102.6 ± 2.9[c]	104.7 ± 2.6[a]	104.6 ± 2.1[d]
Ca	mg/dL	10.1 ± 1	10.2 ± 0.5	10 ± 0.5	9.9 ± 0.8	10.1 ± 0.9	10.1 ± 0.4
P	mg/dL	5.4 ± 1.8	4.8 ± 1.2	5 ± 0.8	4.8 ± 1.6	5.3 ± 1.9	4.4 ± 0.5

[a] $P < 0.05$ compared with male basal-diet group.
[b] $P < 0.05$ compared with male 0.8% Aloe group.
[c] $P < 0.05$ compared with female basal-diet group.
[d] $P < 0.05$ compared with female 0.8% Aloe group.

lesions were also seen with low frequency in the heart, lymph nodes, thymus, parathyroid, nasal cavity, lung/bronchial, salivary gland, stomach, duodenum, urinary bladder, epididymis, ovary, vagina, musculature, skin/subcutis, Zymbal's gland, Harderian gland, and brain.

Table 6 summarizes incidences of neoplastic (benign and malignant) lesions. In colon, adenomas and adenocarcinomas were observed in 4% males (in 4 of 42) and females (3 of 45) but not in any of the 0.8% and control groups. In the cecum, colon and rectum, adenomas, and adenocarcinomas were significantly more frequent in 4% males (in 5 in 42) than in controls. There were no significant differences in the incidences of other tumors between the treated groups and controls of the same genders. Other neoplastic (benign and malignant) lesions were also seen with low frequency in the heart, lymph nodes, parathyroid, adrenal, kidney, testis, ovary, skin/subcutis, Zymbal's gland, eye, abdominal cavity, and brain (data not shown).

No other test-substance related changes were observed.

Table 5—Summary of nonneoplastic or preneoplastic lesions with significant intergroup differences or high frequency incidences.

		Basal diet		0.8% Aloe		4% Aloe	
Organ	**Findings**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
	(Number of rats examined)	38	40	39	42	42	45
Mesenteric lymph node							
	Cellular infiltration, plasma cell	5	0	0[a]	0	1	2
	Dilatation, sinus	5	3	14[a]	7	31[b]	38[d]
Spleen							
	Extramedullary hematopoiesis	27	28	35	29	32	32
Bone marrow							
	Hematopoiesis	6	2	8	6	8	2
Thymus							
	Cyst	1	2	0	2	0	1
	Involution	22	18	25	22	26	29
	Hyperplasia, lymphocyte	0	4	0	0	0	2
Pituitary							
	Cyst	10	0	12	1	9	2
	Pseudocysts	1	0	0	0	1	1
	Hyperplasia	11	5	9	4	11	4
Thyroid							
	Cystic follicles	1	0	0	0	0	0
	Hyperplasia	37	38	39	39	39	39
Adrenal							
	Hyperplasia, cortical	14	3	16	3	20	10
	Hyperplasia, medullary	6	5	3	1	7	3
Cecum							
	Inflammation	0	0	0	0	1	0
	Pigmentation	0	0	1	0	3	0
Colon							
	Pigmentation	0	0	2	1	31[b]	33[d]
	Thickness of epithelium, diffuse	0	0	10[b]	2	34[b]	31[d]
	Atypical hyperplasia	0	0	0	0	5[a]	3
Rectum							
	Pigmentation	0	0	0	0	0	2
	Thickness of epithelium, diffuse	0	0	0	1	0	1
Liver							
	Foci (area) of cellular alteration	11	6	14	9	16	13
Kidney							
	Chronic nephropathy	35	20	34	18	40	12[c]
	Mineralization, cortico-medullary junction	0	22	0	22	0	17
	Mineralization, pelvis	3	4	1	7	2	8
	Pigmentation, tubular	5	18	18[b]	33[d]	36[b]	43[d]
Testis							
	Atrophy	4		2		4	
Prostate							
	Inflammation	13		3[b]		8	
Seminal vesicle							
	Atrophy	5		0[a]		1	
Mammary gland							
	Proliferation, acinar cell	0	15	0	17	0	11
Uterus							
	Hyperplasia		5		6		17[c]
Eye							
	Atrophy of retina	2	5	4	6	5	6

[a] and [b] = significantly different from male control group at $P < 0.05$ and 0.01, respectively.
[c] and [d] = significantly different from female control group at $P < 0.05$ and 0.01, respectively.

Discussion

The present 2-y carcinogenicity study of Aloe at dietary concentrations up to 4% showed no treatment-associated deaths in male and female Wistar Hannover rats, although the survival rate of 4% female group was significantly decreased compared with control females and that of 0.8% females also showed a tendency to increase. Symptoms of diarrhea and reduced body weight gain were evident in both sexes receiving 4%, as in the previous 90-d subchronic toxicity study (Kamiya 2002) and 1-y chronic toxicity study (Matsuda and others 2007), in line with the report of cathartic effects and effectiveness as a laxation of Aloe (Ishii and others 1994). The decrease of survival in the present study might have been associated with body weight loss. Being overweight or obese is a risk factor for many chronic diseases and the impact on well-being and mortality has been well documented (Binns and others 2003; Alley and Chang 2007). Hematological and biochemical examination showed increase of Hb and Pt in the 4% males and RBC, Hb, Alb, and A/G in the 4% females. The cause of these increases could conceivably have been dehydration through diarrhea. There were some changes in other biochemical parameters, AST, Na, K, and Cl, but there was no dose-response in either this study or the previous 1-y study (Matsuda and others 2007). These changes were likely to be also due to dehydration through diarrhea rather than toxicity of Aloe itself. Therefore, they were not considered of toxicological or biological significance.

The severe sinus dilatation and increased yellow-brown pigmentation in ileocecal lymph nodes observed here and in the previous 1-y chronic toxicity study (Matsuda and others 2007) might be due to the inclusion of large amounts of anthraquinone in Aloe. In male Sprague–Dawley rats, the anthraquinone laxative danthron was reported to cause dilatation of the sinusoidal spaces and yellowish pigmentation in lymph nodes of the mesocolon (Sjoberg and others 1988) and, in human cases, anthraquinone laxatives cause melanosis coli and colorectal cancer (Nascimbeni and others 2002; Willems and others 2003). The yellowish pigmentation of kidneys also appeared similar to that in the ileocecal lymph nodes and again has been described earlier and considered as PAS-positive and drug-derived (Sjoberg and others 1988). Although histopathologically diagnosed chronic nephropathy of the kidneys was also seen in both male and female groups in our study, there was no dose-relation and similar lesions were found in the control group.

Incidences of tumors in the cecum, colon, and rectum combined, were here significantly elevated in 4% males, but a conclusion of test chemical carcinogenicity would be paradoxical given the report of antitumorigenic effects to the colon (Shimpo and others 2006). It is reported that suppressive effects of 1% or 5% *Aloe*

Table 6—Summary of neoplastic (benign or malignant) lesions.

		Basal diet		0.8% Aloe		4% Aloe	
Organ	**Findings**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
	(Number of rats examined)	38	40	39	42	42	45
Thymus							
	Lymphoma, malignant, thymic	1	5	4	4	3	3
Pituitary							
	Adenoma, pars distalis	12	30	6	24	8	28
	Adenoma, pars intermedia	2	1	1	0	2	0
	Carcinoma, pars distalis	0	1	0	2	1	1
Thyroid							
	Adenoma, C-cell	2	2	3	3	3	5
	Adenoma, follicular cell	2	1	1	3	3	3
	Carcinoma, C-cell	0	0	0	1	0	0
	Carcinoma, follicular cell	0	0	0	1	1	0
Cecum							
	Adenocarcinoma	0	0	0	0	1	0
Colon							
	Adenocarcinoma	0	0	0	0	1	0
	Adenoma	0	0	0	0	3	3
Rectum							
	Adenoma	0	0	0	0	1	0
Pancreas							
	Adenoma, acinar cell	1	0	1	0	0	0
	Adenoma, islet cell	2	3	0	1	5	2
	Carcinoma, islet cell	1	1	0	0	0	0
Liver							
	Adenoma, hepatocellular	0	0	0	2	2	2
	Carcinoma, hepatocellular	1	0	0	0	0	0
Mammary gland							
	Adenoma	0	3	0	3	0	3
	Fibroadenoma	0	10	0	9	0	10
	Adenocarcinoma	0	1	0	1	0	2
Uterus							
	Adenoma		0		0		1
	Papilloma, squamous cell		0		0		1
	Polyp, endometrial stromal		4		5		7
	Adenocarcinoma		1		0		0

*, ** significantly different from control group 3 at $P < 0.05$, 0.01, respectively.
#, ## significantly different from control group 6 at $P < 0.05$, 0.01, respectively.

arborescens Miller var. *natalensis* Berger on azoxymethane-induced intestinal carcinogenesis in the colon and Aloe may have a chemopreventive effect against colon carcinogenesis at least in the initiation stage. Associations between colorectal cancer risk and anthraquinone laxative use are controversial (Nascimbeni and others 2002), and further examination of colon tumor development in association with diarrhea due to administration of Aloe appears warranted. Mascolo and others have reported that senna pod extract, with anthraquinone and laxative effects, does not cause the appearance of aberrant crypt foci (ACF) or tumors in the rat colon (Mascolo and others 1999). Nor does it have a promoting effect when given to rats at a dose that produces laxation (10 mg/kg), whereas a diarrhogenic dose (100 mg/kg) increased the appearance of tumors induced by azoxymethane (AOM). It has also been well documented that anthraquinones such as 1,8-dihydroxyanthraquinone (DHAQ) and a stimulant named danthron exert tumor promoting effects as well as laxative weak tumorigenic activity in the large intestine of rodents (Mori and others 1986; Sugie and others 1994). Epithelial cell replication with increasing BrdU-labeling indices in the large intestine treated with DHAQ is reported to be associated with altered PGE_2 levels (Nishikawa and others 1997). PGE_2 has also been suggested to exert colon tumor promoting action because of its correlation with ornithine decarboxylase activity (Narisawa and others 1987). The available reports thus suggest possible effects of increased cell proliferation in the colon due to irritation.

Antidiabetic effects of Aloe extract have been reported (Beppu and others 2006) but there was no consistent alterations in blood glucose in the present investigation, or in our earlier 1-y chronic toxicity study (Matsuda and others 2007).

In summary, Aloe, used as a food additive, exerted equivocal carcinogenic potential at 4% high dose level on colon in the present 2-y carcinogenicity study in Wistar Hannover rats. The increase in colon tumors apparent in the 4% groups might have been related to diarrhea caused by the high dose Aloe administration. Taking account of the loss of body weight gain, severe sinus dilatation of ileocecal lymph nodes, and yellowish pigmentation of ileocecal lymph nodes and renal tubules in both sexes receiving 4%, the no observed adverse effect level (NOAEL) for Aloe estimated from the results of the previous 1-y chronic toxicity study (Matsuda and others 2007) was reconfirmed to be 0.16% in both sexes, which is equivalent to 87.7 and 109.7 mg/kg/d in males and females, respectively.

Conclusions

Aloe, used as a food additive, exerted equivocal carcinogenic potential at 4% high dose level on colon in the 2-y carcinogenicity study in rats. Aloe is not carcinogenic at nontoxic-dose levels, based on the previous 1-y chronic toxicity study (Matsuda and others 2007), and that carcinogenic potential at the 4% high-dose level on colon is probably due to irritation of the intestinal tract as a result of diarrhea.

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